

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A recombinant thermostable DNA polymerase which is characterized in that

a) said thermostable DNA polymerase comprises:

i) the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is ~~Glu Arg or Gln~~, and

ii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; and,

b) wherein said polymerase is selected from the group consisting of: *Thermus thermophilus*, *Thermus specie Z05*, *Thermus Specie sps17*, *Thermus caldophilus*, *Thermus filiformis*, *Thermus oshimai*, *Thermus silvanus*, *Thermus chiliarophilus*, *Thermus scotoductus*, *Thermus ruber*, *Thermus brockianus*, *Thermotoga maritima*, *Thermotoga neapolitana*, *Thermosiphon africanus*, *Bacillus caldotenax*, and *Bacillus stearothermophilus*.

2. (Currently amended) The recombinant thermostable DNA polymerase of claim 1 wherein said DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is ~~Glu Arg or Gln~~; and

said nucleotides are dideoxynucleotides and said level of discrimination is at least 3-fold lower than that of said polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu.

3. (Original) The recombinant thermostable DNA polymerase of claim 2 wherein said level of discrimination is measured by determining the concentration of a dideoxynucleotide labeled with a fluorescein dye that is required for 50% inhibition of DNA synthesis.

4-5. (Canceled)

6. (Currently amended) The recombinant thermostable DNA polymerase of claim 1 which is characterized in that

a) said thermostable DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is ~~any amino acid except Glu Arg or Gln~~, and "Xaa" at position 6 is Ser or Ala.

7. (Currently amended) The recombinant thermostable DNA polymerase of claim 1 which is characterized in that

a) said thermostable DNA polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is ~~any amino acid except Glu Arg or Gln~~.

8-10. (Canceled)

11. (Currently amended) A nucleic acid sequence encoding a recombinant thermostable DNA polymerase which is characterized in that

a) said thermostable DNA polymerase comprises:

i) the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid

residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is ~~not~~ Glu Arg or Gln, and

ii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; and,

b) wherein said polymerase is selected from the group consisting of: *Thermus thermophilus*, *Thermus specie Z05*, *Thermus Specie sps17*, *Thermus caldophilus*, *Thermus filiformis*, *Thermus oshimai*, *Thermus silvanus*, *Thermus chliarophilus*, *Thermus scotoductus*, *Thermus ruber*, *Thermus brockianus*, *Thermotoga maritima*, *Thermotoga neapolitana*, *Thermosiphon africanus*, *Bacillus caldotenax*, and *Bacillus stearothermophilus*.

12. (Previously presented) The nucleic acid sequence of claim 11 wherein said nucleotides are dideoxynucleotides and said level of discrimination is at least 3-fold lower than that of said polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu.

13. (Original) The nucleic acid sequence of claim 12 wherein said level of discrimination is measured by determining the concentration of a dideoxynucleotide labeled with a fluorescein dye that is required for 50% inhibition of DNA synthesis.

14-15. (Canceled)

16. (Currently amended) The nucleic acid sequence of claim 12 which is characterized in that

a) said thermostable DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaa~~lle~~ProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is ~~any amino acid except~~ Glu Arg or Gln, and "Xaa" at position 6 is Ser or Ala.

17. (Currently amended) The nucleic acid sequence of claim 12 which is characterized in that

a) said thermostable DNA polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu Arg or Gln.

18-20. (Canceled)

21. (Currently amended) A method of DNA sequencing which comprises:

a) providing a thermostable DNA polymerase characterized in that

- i) said thermostable DNA polymerase comprises:
 - 1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 4 is not Glu Arg or Gln, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; and

ii) ~~wherein said polymerase is selected from the group consisting of: Thermus thermophilus, Thermus specie Z05, Thermus Specie sps17, Thermus caldophilus, Thermus filiformis, Thermus oshimai, Thermus silvanus, Thermus chliarophilus, Thermus scotoductus, Thermus ruber, Thermus brockianus, Thermotoga maritima, Thermotoga neapolitana, Thermosiphon africanus, Bacillus caldotenax, and Bacillus stearothermophilus;~~

- b) providing a dye-terminator labeled with a negatively charged fluorescent dye; and
- c) performing a dye-terminator sequencing reaction.

22. (Previously presented) The method of claim 21 wherein said nucleotides are dideoxynucleotides and said level of discrimination is measured by determining the ratio of the concentration of a dideoxynucleotide labeled with a fluorescein dye required for 50% inhibition of DNA synthesis versus the concentration of an unlabeled dideoxynucleotide required for 50% inhibition.

23. (Original) The method of claim 22 wherein said ratio is 4 or less.

24-25. (Canceled)

26. (Currently amended) The method of claim 21 wherein said amino acid sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is Arg or Gln any amino acid except Glu.

27-30. (Canceled)

31. (Currently amended) A method of producing labeled DNA which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is Arg or Gln any amino acid except Glu, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; and

— ii) — wherein said polymerase is selected from the group consisting of:

~~Thermus thermophilus, Thermus specie Z05, Thermus Specie sps17, Thermus caldophilus, Thermus filiformis, Thermus oshimai, Thermus silvanus, Thermus chiliarophilus, Thermus seotoductus, Thermus ruber, Thermus brockianus, Thermotoga maritima, Thermotoga neapolitana, Thermosipho africanus, Bacillus caldotenax, and Bacillus stearothermophilus;~~

- b) providing a nucleotide labeled with a fluorescein family dye; and
- c) performing a DNA synthesis reaction.

32. (Currently amended) A method of producing labeled primer extension products which comprises:

- a) providing a thermostable DNA polymerase characterized in that

 i) said thermostable DNA polymerase comprises:

 1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is Arg or Gln any amino acid except Glu, and

 2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu;

 3) said polymerase also comprises the second amino acid sequence SerGlnIleXaaLeuArg(Val/Ile) (SEQ ID NO: 18) where "X" is any amino acid except Glu, and

 4) said polymerase has a level of discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; and

— ii) — wherein said polymerase is selected from the group consisting of:

~~Thermus thermophilus, Thermus specie Z05, Thermus Specie sps17, Thermus caldophilus,~~

Thermus filiformis, Thermus oshimai, Thermus silvanus, Thermus chiliarophilus, Thermus scotoductus, Thermus ruber, Thermus brockianus, Thermotoga maritima, Thermotoga neapolitana, Thermosipho africanus, Bacillus caldotenax, and Bacillus stearothermophilus;

- b) providing a ribonucleotide labeled with a fluorescein family dye; and
- c) performing a primer extension reaction.

33. (Currently amended) A kit for DNA sequencing which comprises:

- a) a recombinant thermostable DNA polymerase characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at as position 4 is can be Arg or Gln any amino acid except Glu, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; and

ii) ~~wherein said polymerase is selected from the group consisting of:~~

Thermus thermophilus, Thermus specie Z05, Thermus Specie sps17, Thermus caldophilus, Thermus filiformis, Thermus oshimai, Thermus silvanus, Thermus chiliarophilus, Thermus scotoductus, Thermus ruber, Thermus brockianus, Thermotoga maritima, Thermotoga neapolitana, Thermosipho africanus, Bacillus caldotenax, and Bacillus stearothermophilus;

- b) a terminator labeled with negatively-charged fluorescent dye.

34. (Original) The kit of claim 33 wherein said reduced level of discrimination is measured by determining the ratio of the concentration of ddNTP labeled with a fluorescein family dye required for 50% inhibition of DNA synthesis compared to that for an unlabeled ddNTP and said ratio is 4 or less.

35. (Currently amended) The kit of claim 34 wherein said amino acid sequence comprises: LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is Arg or Gln any amino acid except Glu.

36-38. (Canceled)

39. (Currently amended) A kit for a DNA extension reaction, the kit comprising:

a) a recombinant thermostable DNA polymerase characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at as position 4 is can be Arg or Gln any amino acid except Glu, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; and

ii) wherein said polymerase is selected from the group consisting of:
Thermus thermophilus, Thermus specie Z05, Thermus Specie sps17, Thermus caldophilus, Thermus filiformis, Thermus oshimai, Thermus silvanus, Thermus chiliarophilus, Thermus scotoductus, Thermus ruber, Thermus brockianus, Thermotoga maritima, Thermotoga neapolitana, Thermosiphon africanus, Bacillus caldotenax, and Bacillus stearothermophilus.

40. (Currently amended) The kit of claim 39 wherein said thermostable DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at as position 4 is can be Arg or Gln any amino acid except Glu, "Xaa" at position 6 is Ala

or Ser and "Xaa" at position 7 of said sequence is Ile and said level of discrimination is at least 5-fold lower than that of a thermostable DNA polymerase whose sequence is identical to said thermostable DNA polymerase except that position 4 is Glu.

41. (Currently amended) The kit of claim 40 wherein said recombinant thermostable DNA polymerase is characterized in that the thermostable DNA polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is Arg or Gln any amino acid except Glu.

42-44. (Canceled)

45. (Currently amended) A kit for producing labeled DNA which comprises:

a) a recombinant thermostable DNA polymerase characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is Arg or Gln any amino acid except Glu, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; and

ii) wherein said polymerase is selected from the group consisting of:

Thermus thermophilus, Thermus specie Z05, Thermus Specie sps17, Thermus caldophilus, Thermus filiformis, Thermus oshimai, Thermus silvanus, Thermus chilarophilus, Thermus scotoductus, Thermus ruber, Thermus brockianus, Thermotoga maritima, Thermotoga neapolitana, Thermosiphon africanus, Bacillus caldotenax, and Bacillus stearothermophilus; and

b) a nucleotide labeled with a negatively-charged fluorescent dye.

46. (Currently amended) The kit of claim 45 wherein said amino acid sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO: 3), whereby "Xaa" at position 4 is Arg or Gln any amino acid except Glu.

47-49. (Canceled)

50. (Currently amended) A kit for producing labeled primer extension products which comprises:

a) a recombinant thermostable DNA polymerase which is characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is Arg or Gln any amino acid except Glu, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu;

3) the thermostable DNA polymerase also comprises the second amino acid sequence SerGlnIleXaaLeuArg(Val/Ile) (SEQ ID No: 18) where "Xaa" is any amino acid except Glu;

4) the thermostable DNA polymerase has a level of discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the thermostable DNA polymerase; and

ii) wherein said polymerase is selected from the group consisting of:

~~Thermus thermophilus, Thermus specie Z05, Thermus Specie sps17, Thermus caldophilus, Thermus filiformis, Thermus oshimai, Thermus silvanus, Thermus chiliarophilus, Thermus~~

~~seotoductus, Thermus ruber, Thermus brockianus, Thermotoga maritima, Thermotoga neapolitana, Thermosipho africanus, Bacillus caldotenax, and Bacillus stearothermophilus; and~~
b) a ribonucleotide labeled with a fluorescein family dye.

51. (Currently amended) The kit of claim 50 wherein said amino acid sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is Arg or Gln any amino acid except Glu.

52-81. (Canceled).

82. (New) The recombinant thermostable DNA polymerase of claim 1, wherein position 4 is Arg.

83. (New) The recombinant thermostable DNA polymerase of claim 1, wherein position 4 is Gln.

84. (New) The nucleic acid sequence of claim 11, wherein position 4 is Arg.

85. (New) The nucleic acid sequence of claim 11, wherein position 4 is Gln.

86. (New) The method of claim 21, wherein position 4 is Arg.

87. (New) The method of claim 21, wherein position 4 is Gln.

88. (New) The method of claim 31, wherein position 4 is Arg.

89. (New) The method of claim 31, wherein position 4 is Gln.

90. (New) The method of claim 32, wherein position 4 is Arg.

91. (New) The method of claim 32, wherein position 4 is Gln.

92. (New) The kit of claim 33, wherein position 4 is Arg.

93. (New) The kit of claim 33, wherein position 4 is Gln.
94. (New) The kit of claim 39, wherein position 4 is Arg.
95. (New) The kit of claim 39, wherein position 4 is Gln.
96. (New) The kit of claim 45, wherein position 4 is Arg.
97. (New) The kit of claim 45, wherein position 4 is Gln.
98. (New) The kit of claim 50, wherein position 4 is Arg.
99. (New) The kit of claim 50, wherein position 4 is Gln.